Introduction to Seed Pathology

Seed Pathology

Seed pathology is the branch of pathology that locating to seed and dealing with-

♦ Seed diseases.

♦ Seed borne plant diseases.

♦ Techniques involved for their detection.

♦ Mechanisms of their transmission.

♦ Factors affecting their transmission.

♦ Control measures.

♦ Assessment of inocula, planting value etc.

In modern agricultural science, seed pathology is the branch of plant pathology locating to seed and seed technology rendering (supply) efficient services and opportunities to promote successful agricultural production and quality concerns seed, trade all over the world.

Seed Health

Seed health refers to the presence or absence of diseases causing organisms such as fungi, nematodes, bacteria, viruses and insects and to the status of seeds in a seedlot.

Seed status is also affected by the presence of non disease-causing contaminants in the particular seedlot. These include contaminants like weed seeds that compete with the target seed for nutrients. Other seeds, plant parts other than the target seeds, soil particles and insect eggs that can overwinter can degrade the quality of the seedlot.

Seed health testing

Seed health testing is a procedure by which can be determined whether tile seed is healthy or diseased or it is a procedure by which the presence or absence of seed borne pathogen in a seedlot can be determined.

Objective of seed health testing

1. Seed health testing is necessary for the improvement of seed stock in certification scheme.

2. It is necessary to satisfy quarantine requirements of a country.

3. It is done to know the planting value of a given seedlot in order to forecast the field emergence and predict the health of the mature crops.

4. It is necessary to know the storage quality or feeding value of a seedlot.

5. It is necessary for checking the advisability of treatment.

6. It is done to know the efficiency of seed treating chemicals.

7. To determine the need for drying and processing and specific procedures that should be used.

Methods of Seed health testing

1. Dry inspection method.
2. Microscopic examination of suspension obtained by
    a) Washing test method.
    b) Whole embryo count method.
3. Incubation test-
    a) Blotter method.
    b) Agar plate method.
    c) Deep freezing blotter method.
    d) Water agar plate method.
    e) Test tube agar method.
4. Seedling symptom test-
    a) Hiltner's bricks stone method.
    b) Sand method.
    c) Standard soil method.
    d) Test tube agar method.
5. Growing on test.
6. Serological test.
7. Indicator plant test.
8. Electron microscopy.

▲ Dry Inspection method

It is a very simple and preliminary method for testing the seed health.

Procedure: The dry seed samples were examined for impurities such as;

A) Inert matter: It includes plant debris, spotted, unfilled and Chaffey grains, sclerotia, galls, smut balls, insects etc. It should also be incubated either on blotter or agar media and examined, after a standard period of infection.

B) Symptoms: such as discolouration, staining, necrosis, malformation and similar indication of infection, including fruiting bodies of fungi, resting hyphae in the seed surface, spore or bacterial masses on the seed as well as mechanical damage.

Seed samples are examined first by naked eye and the observed under a steriobinocular microscope for confirmation of the above impurities in proper way. The inert matters need to be incubated for the detection of pathogen associated with these.

Advantage

1. It provides quick information.
2. It does not require much equipment.
3. It helps to take first hand measure.

Disadvantage

1. Only those diseases giving external symptoms and sign can be detected by this method.
2. Information pertaining to viability of seeds cannot be obtained.
3. It is not more reliable because badly infected seeds may look healthy.

Example

Discoloured and spotted rice grains, Black point of wheat, Discoloured and shriveled seed of mustard and jute, Purple stained soybean seed, discoloured seed of white gourd etc can be tested by this method.

▲ Blotter method

The blotter test is a combination of in vitro and in vivo principals of investigation. In this method, the seed are sown in petridish or other suitable containers on moistened absorbent blotting paper, usually three layers to provide enough moisture for duration of the test.

Materials required

1. Plastic petridishes.
2. Blotting paper.
3. Seeds.
4. Sterilised water.
5. Forceps.
6. Stereoscopic microscope.
7. Compound microscope.
8. Cotton.
9. Spirit.
10. Mounting fluid, slide and cover slip etc.

Procedures

1. Required number of plastic petridishes was taken.

2. The petridish were then rinsed with methylated spirit and were dried.

3. The petridishes were left for a while to allow the spirit to be given off.

4. Required number of blotting paper were soaked in sterilised water and three sterilised blotting papers were then placed on each petridish.

5. Then 400 seeds were taken randomly from a working sample.

Surface sterilisation of seedlot

Then the seedlot or working sample were sterilised with 0.001% $HgCl_2$ solution or 10% clorox solution for detection of internal seed borne pathogen. For this, the seedlot was soaked into the 10% clorox solution in a petridish with repeated shaking for about one minute and then the chemical solution were drained out from the petridish carefully. The seedlot was washed with sterilised distilled water for three times to remove the chemicals.

6. 5 or 10 or 25 seeds (with and without surface sterilised) were placed on the wet blotting paper kept at the bottom per petridish or pyrex glass petridish or earthen dish (depending on the size of the seed) maintaining equal distance among the seeds.

7. Then the petridish were kept into an incubation chamber at 20-22^0 C in 12 hours alternating cycles under ultraviolet (UV) light and darkness for 7 days.

Observation

After a week, incubated petridish containing seedlings and non germinated seeds are carefully observed under steriobinocular microscope. The identification and the frequency of different category of fungi associated with the seeds were performed by observing the colour, growth habit and morphological features. The recorded data showing in the following table-

Table: Blotter method of seed health testing

Nature of disinfection	% Germination	% pre emergence death	% post emergence death	No. of colonies	Pathogen (S) associated
Without surface sterilised					
With surface sterilised					

The percentage (%) of healthy seed in the sample tested,

= % Germination - (% pre-emergence death + % post emergence death).

Advantage

1. Pathogen can be detected quickly by observing their growth characters.

2. It is economic.

3. It can be applied for detecting wide range of fungal pathogens from all different kinds of seed.

4. Results obtained by it, more reliable because it is the combination of vitro and in vivo.

5. Blotter method is widely used while agar plate method is impracticable.

Disadvantage

1. Examination may be hampered due to the first growth of certain fungi over the slow growing ones.

2. Pathogenic bacteria cannot be detected.

3. It is time consuming.

4. Pathogenicity cannot be detected.

5. Symptoms may not be detected.

Seed borne Pathogen

Any organism or pathogen carried in or on or with seed, may be termed as seed borne pathogen. e.g. *Curvularia* spp, Loose smut of wheat pathogen, Covered smut of barley pathogen.

Seed Transmitted pathogen

Transfer and re-establishment of seed borne pathogen from seed to plant may be termed as seed transmitted pathogen. These pathogen may be stayed with seed surface, embryo, endosperm etc.

How seed borne pathogen are associated with seeds?

Seed borne pathogen are associated or transmitted by three different ways-

1. Externally seed borne pathogen: The pathogen may be just on the surface of the seeds when such seeds are said to be infested. The inoculum in such cases is superficial and confined to the surface of the seed, usually as adhering propagules. e.g. spore, sclerotia, mycelium, bacteria, nematode, virus particle etc.

Pathogens commonly borne on the surface of seed include species of *Alternaria*, *Fusarium*, many smut, some rust fungi as well as many others.

2. Internally seed borne pathogen: The pathogen may lie within the seed tissues. In such cases, the seeds are said to be infected. Dry seed may look perfectly healthy when examined under a binocular microscope and show no external sign of infection.

Transmission of such pathogens are through vegetative cells, spores, pycnidia, nematode or virus particles. For example,

Ustilago tritici (loose smut of wheat).

Ustilago nuda (covered smut of barley).

Xanthomonas campestris (bacterial blight of cotton).

3. Concomitant contamination: Besides seed infection and seed infestation there can be brought another kind of association of pathogens with seed materials, it is called concomitant contamination. In this case the seed material is mixed with bits of infected crop tissues. For example, Bajra seed mixed with downy mildew. Infected bajra leaf bits containing oospores of the pathogen or mixed with microscopic part of the pathogen.

e.g. Sclerotia of ergot of bajra.

Importance of seed borne diseases in Bangladesh

Bangladesh is an agro based country. The principal crops are Rice, Wheat, Jute, Potato, Sugarcane, Pulses, Oil seeds and different vegetables. Most are the crops are affected by seed borne diseases. In Bangladesh more than 400 seed borne diseases are recorded to attack 72 different crop plants. The plant pathogen like fungi, bacteria, viruses, nematode are responsible for this. Seed borne diseases cause enormous losses to crop every year in Bangladesh. The losses are occurred both in the field as well as in storage.

In field condition, sowing of the seeds attacked by seed borne diseases, creates failure of germination due to the activation of pathogens. As a result, the total crop losses occurred. If some healthy seeds are germinated, the other affected plants act as a source of infection for the healthy seedlings. Sometimes the virgin soil is attacked because some pathogens are able ubiquitous (both seed borne and soil borne). The crops which are attacked in the field condition due to seed borne diseases are described below-

1) Rice: It suffers from 17 different seed borne diseases. In 1943, the Bengal famine was occurred due to the Brown spot of rice disease which is seed borne disease. The other seed borne rice diseases are blast, bakanae, stem rot etc. which sometimes cause enormous losses to rice crop.

2) Wheat: It is attacked by different seed borne diseases. e.g. Loose smut, Leaf blight, False smut, Seed gall (nemic disease) etc.

3) Jute: It has many seed borne disease but stem rot, anthracnose and mosaic of jute are most important. Every year, jute production are losses due to these disease.

4) Potato: The most important and damaging seed borne disease of potato are late blight (*Phytopthora infestans*), virus diseases (Leaf roll, Potato virus X, potato virus Y). In favourable weather condition, these disease can cause huge losses to potato [The greater Irish Famine was occurred due to the late blight of potato disease].

5) Sugarcane: Among the seed borne diseases of sugarcane, Red rot (*Colletotrichum falcatum*) and white leaf (mycoplasma) are most serious. At least 10% sugarcane production is affected due to this disease.

6) Pulses: Foot and root rot, Ascochyta blight etc. are main seed borne diseases.

7) Oil seeds: Alternaria of mustard, collar rot of sunflower, Tikka disease of groundnut, Anthracnose are main seed borne diseases in oil seed crops.

8) Vegetables: Major seed borne diseases in vegetable are bean anthracnose, rust, mosaic of okra, Fusarium wilt etc.

A rough estimate made in 1989 shows that annual loss of about tk. 900 crores is occurred due to different seed borne disease occurring only in field crops in Bangladesh.

9) In storage condition: Considerable losses also occur due to the invasion of seed borne microorganisms in storage condition. It has been found that species of *Aspergillus* and *penicillium* are responsible for the deterioration of stored seeds of rice, wheat, pulses, oil seeds and different vegetables crop. In the opinion of the experts, an average 2-4% of the total stored seeds are destroyed by the activities of storage fungi in the country. Approximately 10-15% of the total vegetatively propagated seeds used both for sowing and consumption are lost annually in storage due to seed borne diseases. Thousand tons of potato are lost in every year in storage condition due to dry rot (*Fusarium* spp.), black heart and storage disease.

Significance of seed transmission of pathogen

Seed transmission is the act of dissemination of pathogen and inoculation of host by the pathogen through seeds for the preparation of disease from one generation to another generation.

Plant pathogen can be transmitted through various agents. Among them seed is the most efficient carrier of pathogen. The significance of transmission of pathogen through seed are discussed below-

1. Dormant conditions: The dormant conditions of the seed is highly favourable for the survival of pathogens in it. As a result, pathogen will remain alive in seed for long time.

2. Small amount: A small amount of seed or its structure can able to carry pathogen.

3. Virgin soil: Virgin soil may be inoculated through infected seeds and can persist long time.

4. Intimate association of host and parasites: The intimate association of host and parasites both in space and time, provides maximum opportunity for the earliest infection of the emerged seedlings.

5. Germination failure of the seed: Infected seeds fail to germinate or the young seedlings emerging from the infected seeds die after germination resulting pre-emergence death, damping off, seedling blight.

6. Providing primary sources of inoculum: It provide primary sources of inoculums in the field. The primary inocula grow, germinate and multiplying on the infected dead seeds and seedlings. Later on these inocula may be transmitted to the healthy plants of some or neighbouring field and can cause disease often in epidemic form.

Not infected → germinate multiply → some plant infected → neighbouring field (all infected)

7. Providing numerous foci of inoculum: It provides numerous foci of primary infection. Thus rendering the crop of the entire field vulnerable to the attack of the pathogen.

8. Pathogen can be spread over a long distance through seeds: A pathogen can be spread over long distance through infected seeds. On this way a new or unknown pathogen or a virulent race of a known pathogen can be introduced from one village to another village or from one distance to another distance or even from one country to another.

9. Resistant cultivars converted into susceptible: Seed borne inoculum obtain the presence of a virulent race/strain of the pathogen along with the seed. Thus new aggressive pathological races of pathogens maybe introduced with the seed. As a result, the varieties of resistant to cultivate to local races of the pathogens may become susceptible to the newly introduced aggressive races.

Condition on which transmission of pathogen depends

1. Growth stage of the host.
2. Paths of entry of pathogen into the plant or seed.
3. Weather conditions.
4. Flowering period of the host.
5. Localisation of pathogen into the seed.

All plants are not seed borne; Why?

All pathogen are not seed borne because seeds are protected by almighty Allah. However, there are following reasons behind this-

1. Many pathogen cannot invade ovule through plasmadesma connection.

2. When ovule is invaded by pathogen, the ovule may get shocked and that is why the seed may die. i.e. abortion occurs.

3. Just after fertilisation, the cells of embryo divide so quickly that the pathogen may die.

Entry point of seed infection

A. Infection directly from the mother plant.

B. Infection from outside.

a) Intraembryal infection through stigma.

b) Extraembryal infection through stigma.

c) Ovary wall, pericarp and integuments of seed coat as path of infection.

d) Flower and fruit stalk as path of infection.

Difference between Seed borne pathogen and Seed transmitted pathogen

Seed borne pathogen	Seed transmitted pathogen
1. Any pathogen carried in or on or with the seed may be termed as seed borne pathogen.	1. During transfer and re-establishment of seed borne pathogen from seed to plant, then pathogen may be termed as seed transmitted pathogen.
2. All seed transmitted pathogens are seed borne pathogen.	2. All seed borne pathogens may not seed transmitted pathogen.
3. Pathogen must be established.	3. Pathogen may or may not be established.
4. Carrying and establishment pathogen into the seed occurs.	4. Seed just a vehicle of pathogen.
5. Example: *Alternaria* spp	5. Example: *Ustilago tritici*.

Why Seed borne diseases of crop are important?

1. Seeds can carry these pathogens through time and space.

2. Plants can hardly escape seed transmitted pathogens.

3. Most of the major plant diseases are seed borne.

4. Seeds may carry dangerous pathogen to a region or country where it was not there such introduction may become catastrophic.

5. High economic significance.

Mechanism of seed transmission of pathogen

The mechanism of seed transmission of pathogen refers to transfer of a pathogen from seed to plant and again to seed. In nature, it is a highly complicated process. The entire process consist of at least 3 different phases-

A. Entry of pathogen into seed.

B. Location of pathogen in the seed.

C. Transmission of pathogen from seed to plant to seed.

A. Entry of pathogen into seed: The pathogen enter into seed by different ways-

1. Entry of pathogen directly from mother plant. e.g. *Fusarium*, *Verticillium*. These pathogens invade seed through funiculus.

2. Entry of pathogen from outside of the mother plant.

i) Through stigma. e.g. *Ustilago tritici*.

ii) Through pericarp. e.g. *Rhizoctonia solani* in chilli seeds.

iii) Through the flower and fruit stalk. e.g. *Colletotrichum lini*.

iv) Through the seed coat. e.g. *Alternaria*, *Cercospora* etc.

B. Location of pathogen in the seed: The pathogen may be located in the following parts of the seed-

a) Ovule: *Fusarium culmorum* in wheat seeds.

b) Embryo: *Ustilago tritici*, *Ustilago nuda* in wheat seed.

c) Endosperm: *Drechslera oryzae*, *Bipolaris sorokiniana*.

d) Seed coat: Most of the Dematiaceous, Hyphomycetes and Coelomycetes fungi.

e) Seed surface: *Ustilago hordei* in barley seeds, propagules of fungi may be present in seed surface as concomitant contamination.

f) Bracts, glumes and other seed parts: *Puccinia malvacearum*, *Cercospora beticola* in beet and hollyhock seeds.

C. Transmission of Pathogen from seed to plant to seed

Pathogen can be transmitted from seed to plant again to seed by various ways.

According to Paul Neergard (Father of seed pathology) at least 8 different categories of seed-plant-seed transmission are well recognized. They are as follows:

1. Intraembryal infection followed by systemic infection.

2. Intraembryal infection followed by local infection.

3. Extraembryal infection followed by systemic infection.

4. Extraembryal infection followed by local infection.

5. Seed contamination followed by systemic infection.

6. Seed contamination followed by extramatrical saprophytism or a dormant stage and thereafter by local infection.

7. Seed contamination followed by extramatrical saprophytism and systemic infection.

8. Seed contamination by structures from organ specific seed infection followed by an extramatrical non-parasitic phase and later by direct organ specific infection.

1. Intraembryal infection followed by systemic infection: e.g. *Ustilago tritici* in wheat.

The pathogen remains inside the embryo and become activated in favourable conditions during seed germination. Then proceeds systematically and infected every parts of the plant during its growth. But in case of *Colletotrichum dematium*, the symptom express in the cotyledon; after some days this cotyledon are fallen down.

2. Intraembryal infection followed by local infection: e.g. *Ascochyta pisi* in pea.

i) Embryo is infected.

ii) Pathogens become active by the germination of seeds and causes primary infection.

iii) The pathogen is spread from primary infection to leaves, petioles and stems to produce local lesions.

3. Extraembryal infection followed by systemic infection: *Drechslera graminea* in barley.

i) The pathogen remain outside the embryo.

ii) When the seed germinate, the pathogen will germinate and multiply. Then infect systematically through coleoptile. Symptom is expressed in the whole plant. Blight symptom is produced in cereals and head infection caused by botrytis.

4. Extraembryal infection followed by local infection: e.g. *Alternaria brassicicola*, *Xanthomonas phaseoli*.

The pathogen remain outside the embryo which under multiplication infect the upper leaves and then pod (siliqua). Inside the siliqua grain formation is infected. But *phaseoli* infect leaves and pod.

5. Seed contamination followed by systemic infection: e.g. *Ustilago hordei*, *Tilletia caries*.

The pathogen remain outside the seed surface. During seedling stage the pathogen remain as teliospore. Later on germination, it produce basidia and spores then carried by air and infect the plant systematically. Main symptoms develop in the head and mild symptom in the upper leaf.

6. Seed contamination followed by extrametrical saprophytism or a dormant stage and thereafter by local infection: e.g. *Sclerotinia sclerotiorum*.

The pathogen remain outside the seed. i.e. In soil. When irrigation is added, the pathogen (sclerotium) germinate and infect young seedlings locally. Main infection occur at seedling stage. The entire plant may wilted and foot rot occur at collar region.

If attack later stages of plant growth, the sclerotium remain viable in the folded leaf or with in the foliar parts and again seed is infected.

7. Seed contamination followed by extrametrical saprophytism and systemic infection: e.g. *Fusarium oxysporum*.

The pathogen remain viable in the form of conidia or mycelial fragment. When seed is germinate, the conidia also germinate. The crust of conidia are found at the base of the conidia. These become disseminated by irrigation water or insect or other factors on the upper parts. Then infect systematically and develop symptom of foot rot and wilt. In wilting condition, no spot is occurred.

8. Seed contamination by structures from organ specific seed infection followed by an extrametrical non-parasitic phase and later by direct organ specific infection:

e.g. *Claviceps purpurea*, *Anguina tritici*.

The pathogen is organ specific. Directly infect to the grain of panicle and ovary. Then replaced it, then fall on the field. Again they germinate and produce drum stick like conidiophore on maturity. They are disseminated and infect again.